Allergenicity of grass pollen from polluted and non-polluted areas measured by skin prick test and basophil activation test

Alfaya Arias T1,2, Feo Brito F1,3, Somoza Álvarez ML 4, Amo-Salas M 5, Lucas JA6, Gutiérrez Mañero J6, Urra Ardanaz JM 7

1Allergy Department. Hospital General Universitario de Ciudad Real, Spain
2Allergy Department. Hospital Universitario Fundación Alcorcón, Madrid, Spain
3Faculty of Medicine. Universidad de Castilla-La Mancha (UCLM), Spain
4Allergy Department. Hospital Universitario Infanta Leonor, Madrid, Spain
5Faculty of Medicine. Mathematics department. Universidad de Castilla La Mancha (UCLM), Spain.
6Plant Physiology, Pharmaceutical and Health Sciences Department, Faculty of Pharmacy, Universidad San Pablo-CEU, Spain
7Immunology Department, Hospital General Universitario de Ciudad Real. Spain

Corresponding author:
Teresa Alfaya Arias
Allergy Department. Hospital Universitario Fundación Alcorcón
C/ Budapest s/n, 28922, Alcorcón, Madrid, Spain.
E-mail: teresa.alfaya@salud.madrid.org

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0775
Pollution has been associated with pulmonary diseases including asthma. The bronchial inflammation caused by the contaminants increases the effect of airborne allergens in susceptible patients [1].

Asthmatic patients allergic to pollen who reside in cities with high levels of industrial pollution have a worse disease progression and more exacerbations than patients who live in cities with lower levels of pollution [2,3]. Furthermore, industrial pollution has been linked to high enterobacteria counts in grass pollen and increase in the release of endotoxins amplifying the immune response and inflammation of the airways when pollen is inhaled [4].

Furthermore, atmospheric pollution may affect plants and their pollen. Several studies have found an increase in the allergenicity of pollen from plants and trees exposed to different contaminants. However, most studies have been performed under laboratory conditions and few studies have focused on grass pollen [5-10].

Previous studies by our group have shown that pollution is associated with a reduction in photosynthetic efficacy and a higher degree of oxidative stress in grass plants and pollen proteins [8].

In the present study, we analyze the allergenicity of grass pollen from a large city with high levels of environmental pollution (Madrid) and a city with low levels of pollution (Ciudad Real) under conditions of real environmental exposure of the plants in their habitat and not in a laboratory setting.

The study was approved by the Ethics and Research Committees of the participating hospitals. All patients gave their written informed consent to participate.

Statistical analysis was performed by the SPSS software package. For the comparison of the means of quantitative variables the Student-t test was used when the data followed a normal distribution and Mann-Whitney test was used when data didn’t follow it.

Madrid and Ciudad Real are located in the center of Spain. Both have a dry continental climate with short periods of grass pollination about 6 weeks in May and June.

Levels of contaminants and weather data were obtained from the City Council of Madrid and Regional Government of Castilla la Mancha websites. Data were collected from
October to May for the years 2017, 2018 and 2019 as this is the period which affects grass pollen formation.

After data analysis we found significantly higher levels of NO\textsubscript{2} and SO\textsubscript{2} in Madrid. There were no significant differences in temperature nor precipitation between Madrid and Ciudad Real (Online Supplemental files).

With regard to pollen counts, from 1st May to 30th June, daily pollen concentrations were measured in Madrid (Hospital Infanta Leonor) and Ciudad Real (Hospital General de Ciudad Real) using a Burkard spore-trap (Burkard Manufacturing Co., Rickmansworth, Herts, UK) as described before [2,3]. No significant differences in grass pollen counts were found between both cities (Online Supplemental files).

During the pollination period (May) of the years 2017, 2018 and 2019 Lolium perenne plants were collected in Madrid and Ciudad Real from areas near roads with vehicle traffic. The mature pollen was extracted by suction as described before [8].

Skin prick tests were performed on a sample of 75 patients with allergic rhinitis or asthma and sensitization to grass pollen, outside the grass pollen season using each original extract from the pollen collected in Madrid and Ciudad Real. 53.3% of the patients were female and the median age was 26 years. The mean wheal diameter was significantly greater with the pollen collected in Madrid (Table 1).

Basophil activation test (BAT) with both pollen extracts, expressed as the percentage of CD63\textsuperscript{+} basophils, was determined using a BASOTEST kit (Glyco-type Biotechnology GmbH, Heidelberg, Germany). The BAT was performed in 8 patients with respiratory allergy due to grass pollen. The mean percentage expression of CD63 (activated basophils) was slightly higher with the pollen from Madrid (Table 1) although the difference was not statistically significant.

In this study of grass plants collected in Madrid and Ciudad Real in their natural environment we have found that the grass pollen from Madrid produces a greater wheal size in the skin tests performed on allergic patients. Given that pollen levels, temperature and humidity are similar in both cities, we attribute this effect to the higher levels of the contaminants NO\textsubscript{2} and SO\textsubscript{2} in Madrid. However, we have not been able to demonstrate significant differences in the BAT using both pollens, probably due to the small number of patients included in this arm of the study.

Some studies have found an increased allergenicity in pollen exposed to pollutants as CO, O\textsubscript{3}, NO\textsubscript{2} and SO\textsubscript{2} under laboratory conditions [5-10]. However Smiljanic [9] found that pollen from Phleum pratense collected from highly polluted areas expressed lower levels of Phl p 6, 12 and 13 and showed with ELISA less binding to IgE in allergic patients.

Ghiani [7] conducted a study with Ambrosia pollen exposed naturally to environmental pollution, finding that pollen exposed to high levels of traffic pollution was more allergenic in SDS–PAGE and immunoblotting.
Most of these studies are based on in vitro tests with serum from patients, but García Gallardo [10] found that *Pinus radiata* pollen collected in areas of high concentrations of O₃ yielded more positive results also in skin prick tests.

In conclusion, in this study we collected pollen from grass plants exposed naturally to contaminants and analyzed the response in vivo using skin tests and, as a novel feature, also in vitro using the BAT. Skin tests revealed the greater allergenicity of the grass pollen exposed to contaminants as compared to that not exposed to such levels of pollution.

Environmental pollution constitutes an added risk factor for patients allergic to pollen, not only due to its direct proinflammatory action on the airways but also because it increases the allergenicity of the pollen itself.

**Presentation in Congress**
Data have been presented in electronic poster form at 33 Congres of Sociedad Española de Alergología e Inmunología Clínica (SEAIC) in October 2021.

**Founding Sources**
Ministerio de Economía y Competitividad, Instituto de Salud Carlos III of Spanish Government PI15/00715, PI18/00095, and co-founded by Fondo Europeo de Desarrollo Regional—FEDER for the Thematic Networks and Co-operative Research Centres: ARADyAL (RD16/0006/0028, RD16/0006/0024 and RD16/0006/0001).

**Conflicts of Interests**
The authors declare no conflicts of interests

**References**


Table 1. Skin prick tests (mean diameter: major x orthogonal diameter) and Basophil activation test (percentage of CD63+ basophils) with grass pollen extracts from Madrid and Ciudad Real.

<table>
<thead>
<tr>
<th></th>
<th>POLLEN A (CIUDAD REAL)</th>
<th>POLLEN B (MADRID)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SKIN PRICK TEST (mm) n=75 * p&lt;0.01</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>35,44</td>
<td>43,69</td>
</tr>
<tr>
<td>Median</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>Stand. Desv.</td>
<td>24,44</td>
<td>31,24</td>
</tr>
<tr>
<td>Min</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Max</td>
<td>130</td>
<td>150</td>
</tr>
<tr>
<td><strong>BASOPHIL ACTIVATION TEST (%CD63) n=8</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>56,25</td>
<td>60,58</td>
</tr>
<tr>
<td>Median</td>
<td>59,35</td>
<td>70,7</td>
</tr>
<tr>
<td>Stand. Desv.</td>
<td>35,17</td>
<td>32,55</td>
</tr>
<tr>
<td>Min</td>
<td>9</td>
<td>13,8</td>
</tr>
<tr>
<td>Max</td>
<td>95,7</td>
<td>93</td>
</tr>
</tbody>
</table>